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Your reference: HL83615/001

Patent application number *(The Patent Office will fill in this part)*: 22 JAN 2003 0301505.4

Full name, address and postcode of the or of each applicant *(underline all surnames)*: Phytopharm plc
Corpus Christi House, 9 West Street,
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Patents ADP number *(if you know it)*: 05838123003

If the applicant is a corporate body, give the country/state of its incorporation: United Kingdom

Title of the invention: Stereospecific Synthesis of Sapogenins

Name of your agent *(if you have one)*: Haseltine Lake

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*:
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Patents ADP number *(if you know it)*: 34001

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11. I/We request the grant of a patent on the basis of this app

Signature Rosalthie Lake. Date 22/1/0

12. Name and daytime telephone number of person to contact in the United Kingdom — Mr. David L Brown — [0117] 910 3200

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STEREOSPECIFIC SYNTHESIS OF SAPOGENINS

Field of the Invention

The present invention relates to the stereospecific synthesis of sapogenins.

Background of the Invention

It has been shown that certain sapogenins and their pro-drugs possessing a 5β-hydrogen atom (more particularly, the so-called A/B-cis sapogenins which have 5β,10β stereochemistry, such as sarsasapogenin, episarsasapogenin, smilagenin and epismilagenin) have utility in the treatment of cognitive dysfunction and other conditions. Such activity is described, for example, in WO-99/48482, WO-99/48507, WO-01/49703, WO-02/079221 and WO-01/23406, the disclosures of which are incorporated herein by reference.

Episarsasapogenin has also found utility as a raw material for the synthesis of Eltanolone as described in WO-98/07741.

The present invention has as an object the stereospecific synthesis of such sapogenins, and more preferably the 5β-sapogenins defined and described in the said publications, as well as their prodrugs and other physiologically acceptable forms such as salts, with stereospecific control of the 3-position atoms or groups.

Most preferably, the present invention has as an object an efficient stereospecific synthesis of sarsasapogenin, episarsasapogenin, smilagenin or epismilagenin or their pro-drugs and other physiologically acceptable forms.

US Patent No. 3,875,195 (1975), the disclosure of which is incorporated herein by reference, describes the catalytic reduction of 3-keto-5β-H steroids to 3β-hydroxy-5β-H steroids in a lower carboxylic acid with Raney nickel and hydrogen under pressure.

Since the introduction of the family of highly hindered trialkylborohydride reducing agents, commonly known as selectrides®, beginning in the early 1970s (Brown et al., *J. Am. Chem. Soc.* 94, 7159-7161 (1972)), a number of publications

have appeared in which these reducing agents have been applied to certain sterol synthetic methods.

For example, in *Steroids*, 36, 299-303 (1980), *Steroids*, 45,39-51 (1985), *J. Chem. Soc. Commun.* 1239-1240 (1982), *Tetrahedron*, 40, 851-854 (1984), *Helv. Chim. Acta*, 66, 192-217 (1983), and *Tetrahedron*, 45, 3717-3730 (1989), the disclosures of which are incorporated herein by reference, stereospecific selectride reductions of certain 3-keto-5β and 3-keto-5α steroids to their respective 3β-OH, 5β-H and 3α-OH, 5α-H sterols are described.

Diborane has been reported as a reducing agent capable of stereospecifically reducing 5β-cholestan-3-one to 5β-cholestan-3α-ol (*Steroids*, 34, 121-124 (1979)).

In relation to sapogenins, the art has reported catalytic acid hydrogenation, as exemplified by Blunden's preparation of epitigogenin from tigogenone using hydrogenation over an Adams catalyst (platinum (IV) oxide) in glacial acetic acid containing 2% hydrochloric acid (*J. Nat. Prod.* 42, 478-482 (1979); *Onderstepoort J. Vet. Res.*, 61, 351-359 (1994)). The art has also reported sodium borohydride reduction, as exemplified by Miles's preparation of episarsasapogenin (in 86% yield after column chromatography) from sarsasapogenone using sodium borohydride (*J. Agric. Food Chem.*, 41, 914-917 (1993)). The art has also reported lithium aluminium hydride reduction, as exemplified by Djerassi's preparation of epismilagenin (in 73% yield after several recrystallisations from methanol-chloroform) from smilagenone using lithium aluminium hydride (*J. Am. Chem. Soc.*, 74, 422-424, (1952)).

The present invention now enables sapogenins and their pro-drugs and other physiologically acceptable forms to be prepared with improved efficiency and with controllable stereospecificity at the 3-position.

Brief Description of the Invention

The present invention provides in a first aspect a method of stereospecifically preparing a sapogenin which comprises reducing a sapogen-3-one using as reducing agent: a selectride; an organo-aluminium hydride; aluminium hydride

(AlH_3); a borane; hydrogen and a catalyst; or an alkali metal borohydride and cerium halide.

The term "selectride" as used herein means any alkali metal tri-alkyl or tri-aryl borohydride reducing agent, from any manufacturer or source, such as lithium tri-*sec*-butylborohydride, lithium trisamylborohydride or lithium triphenyl borohydride, or the corresponding reducing agents with lithium replaced by potassium or sodium. Alkyl groups preferably contain from 1 to 7 carbon atoms. Aryl groups preferably contain from 6 to 12 carbon atoms and may be alkyl-substituted. For a more detailed discussion, refer to "Reductions by the Alumino- and Borohydrides in Organic Synthesis", by J. Seyden-Penne (VCH Publishers, Inc.). Preferred selectrides for use in the present invention are L-selectride® (lithium tri-sec-butylborohydride), K-selectride®. (potassium tri-sec-butylborohydride), N-selectride® (sodium tri-sec-butylborohydride), LS-selectride® (lithium trisamylborohydride), KS-selectride® (potassium trisamylborohydride) and potassium or lithium triphenyl borohydride.

The term "organo-aluminium hydride" means any reducing agent containing aluminium and hydride moieities and organic groups (e.g. alkyl or alkoxy, suitably containing from 1 to 7 carbon atoms), such as sodium bis(2-methoxyethoxy) aluminium hydride (Red-Al®), di-isobutyl aluminium hydride (DIBAL) or lithium aluminium tri-*tert*-butoxy hydride (LTBA). For more detailed discussion, refer to "Reductions by the Alumino- and Borohydrides in Organic Synthesis", by J. Seyden-Penne (VCH Publishers, Inc.). Preferred organo-aluminium hydride for use in the present invention are Red-A, DIBAL and LTBA.

The borane is preferably diborane.

The catalyst for the hydrogenation is preferably palladium or platinum.

By appropriate selection of the reducing agent the method potentially enables a full range of 3α,5β, 3α,5α, 3β,5β, and 3β,5α sapogenins to be prepared in substantially stereoisomerically pure form in good or excellent overall yield from a commercially available or readily preparable starting material, with avoidance of difficult separation of isomer mixtures and by a synthetic route in which all side

products can be held in the reaction mixture until the end product has been obtained.

Wherein the reducing agent is a selectride and the starting material is a 5β-sapogen-3-one, the sapogenin obtained may predominantly be a 3β,5β sapogenin.

Wherein the reducing agent is a selectride and the starting material is a 5α-sapogen-3-one, the sapogenin obtained may predominantly be a 3α,5α sapogenin.

Where the reducing agent is an organo-aluminium hydride or hydrogen and a catalyst and the starting material is a 5β-sapogen-3-one, the sapogenin obtained may predominantly be a 3α,5β sapogenin.

The expression "sapogen-3-one" used herein is used herein for convenience, to refer to the staring material for the reduction, and does not necessarily imply saturation outside the A ring.

The sapogen-3-one starting material may suitably be prepared by oxidation of the corresponding sapogenin. For example, sarsasapogenone has been prepared by oxidation with pyridinium dichromate as described by Miles (J. Agric Food Chem., 1993, **41**, 914-917), Jones oxidation as described by Blunden (J. Nat. Prod., 1979, **42**, 478-482) and in WO-98/07741. Tigogenone may similarly be prepared by a Jones oxidation of tigogenin (J. Nat. Prod., 1979, **42**, 478-482). Smilagenone is commercially available form Steraloids Inc.

The method of the present invention is preferably used for the preparation of a sapogenin selected from sarsasapogenin, episarsasapogenin, smilagenin and epismilagenin. Pro-drugs and other physiologically acceptable forms of the sapogenins may be prepared from the 3-OH compounds in conventional manner, as described in more detail below.

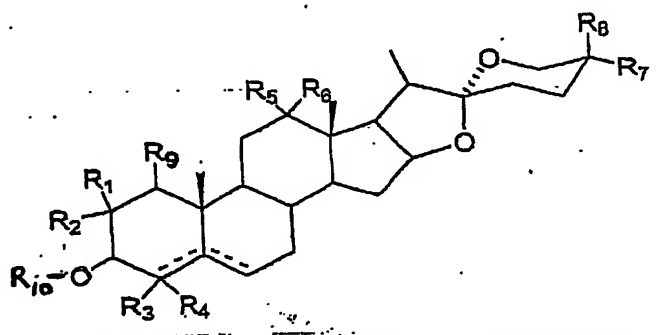
In a second aspect of the invention therer is provided the conversion of 3α-hydroxy-5β-sapogenins to 3β-hydroxy-5β-sapogenins and esters thereof, by a stereospecific inversion reaction. A suitable stereospecific inversion reaction is the Mitsonubu reaction, details of which are found in, for example, Hughes,

Organic Reactions, 1992, 42, 337-400, the disclosure of which is incorporated herein by reference.

Detailed Description of the Invention

The Desired Sapogenin End Products

The method of the present invention is preferably used to prepare sapogenin end products selected from compounds of general formula (I):



wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are, independently of each other, H, C_{1-4}–alkyl, OH or OR, where R = C_{6-12}–aryl or C_{1-4}–alkyl; or R_5 and R_6 together represent a protected carbonyl group;

---- signifies an optional double bond;

the stereochemistry at the 3 position carbon centre can be either R or S;

and R_{10} is H or any organic ester group (which includes aliphatic and aminoacid esters);

including all stereoisomers thereof and racemic mixtures where the stereochemistry of general formula I permits, their physiologically acceptable prodrugs and salts.

Most preferably, the desired sapogenin end product is one of sarsasapogenin, episarsasapogenin, smilagenin and epismilagenin, or a physiologically acceptable pro-drug or other physiologically acceptable form (e.g. salt) thereof.

The term "physiologically acceptable prodrug" as used herein means those prodrugs of the compounds useful according to the present invention which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic

response, and the like, commensurate with a reasonable benefit/risk ration, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" means compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formula, for example by hydrolysis in blood. Functional groups which may be rapidly transformed, by metabolic cleavage, in vivo form a class of groups reactive with the carboxyl group of the compound of this invention. Because of the ease with which the metabolically cleavable groups of the compounds useful according to this invention are cleaved in vivo, the compounds bearing such groups act as pro-drugs. A thorough discussion of prodrugs is provided in the following: Design of Prodrugs, H. Bundgaard, Examining Division., Elsevier, 1985; Methods in Enzymology, K. Widder et al, Examining Division., Academic Press, 42, p.309-396, 1985; A Textbook of Drug Design and Development, Krogsgaard-Larsen and H. Bundgaard, Examining Division., Chapter 5; Desing and Applications of Prodrugs p.113-193, 1991; Advanced Drug Delivery Reviews, H. Bundgaard, 8, p.1-38, 1992; Journal of Pharmaceutical Sciences, 77.p285, 1988; Chem. Pharm. Bull., N. Nakeya et al, 32, p.692, 1984; Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, Vol. 14 of the A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, Edward B. Roche, Examining Division. American Pharmaceutical Association and Pergamon Press, 1978, which are incorporated herein by reference.

The term "physiologically acceptable salts" as used herein means the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. See, for example S. M. Berge, et al., Pharmaceutical Salts, J. Pharm. Sci., 66: p.1-19 (1977) which is incorporated herein by reference. Base addition salts can also be prepared by separately reacting the purified compound in its acid form with a suitable organic or inorganic base and isolating the salt thus formed. Base addition salts include pharmaceutically acceptable metal and amine salts.

The term "protected carbonyl group" used herein refers to a C=O group which has been reacted with an agent which serves to protect reactive functional carboxy

groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional carbonyl protecting agents may be used in accordance with standard practice, for examples see T.W. Green and P.G.M. Wuts in "Protective Groups in Organic Chemistry" John Wiley and Sons, 1991; J.F.W McOmie in "Protective Groups in Organic Chemistry" Plenum Press, 1973, the disclosures of which are incorporated herein by reference.

The sapogen-3-one

The sapogen-3-one, the starting material for the step which results in the preparation of the desired sapogenin, preferably corresponds to the desired sapogenin at all points of the molecule except the 3-position group. However, if necessary or desirable, suitable protective groups may be applied for the reduction, and subsequently removed to yield the desired sapogenin.

The term "protective groups" used herein refers to groups which are used to protect reactive functional groups, for example hydroxy or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice, for examples see T.W. Green and P.G.M. Wuts in "Protective Groups in Organic Chemistry" John Wiley and Sons, 1991; J.F.W McOmie in "Protective Groups in Organic Chemistry" Plenum Press, 1973.

The compound thus prepared may be recovered from the reaction mixture by conventional means. For example, the compounds may be recovered by distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water, followed by extraction with a water-immiscible organic solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well known techniques, such as recrystallization, re-precipitation or the various chromatography techniques, notably column chromatography or preparative thin layer chromatography.

Reduction of the sapogen-3-one

We have found that either a 3α or a 3β hydroxyl could be formed by the correct choice of reducing agent. A number of reagents have been discovered to effect selectivity to either afford smilagenin or epismilagenin, as shown in Table 5 below. Surprisingly we have found that the use of K-, L- or N-selectride® (potassium, lithium or sodium tri-*sec*-butylborohydride) leads to the formation of the 3β-hydroxyl, e.g smilagenin, in a highly stereoselective manner.

We have also made the surprising discovery that the use of lithium tri-*tert*-butoxyaluminium hydride leads to the formation of the 3α-hydroxyl, e.g epismilagenin, in a highly stereoselective manner.

Table 1 below lists reagents used in the reduction of smilagenone.

In the stereoselective reduction of sapogen-3-ones according to the present invention, we have found that it is possible to obtain in the end product a molar ratio of the predominant 3-hydroxy steroid obtained, to the alternative 3-epimer, of at least about 10:1, for example at least about 15:1.

Table 1 - Selectivity in the reduction of smilagenone.

Reagent	Temp/°C	Solvent	Smilagenin/%	Epismilagenin/%	Smilage
$LiAlH(O^tBu)_3$	RT	THF	5.0	95.0	-
$LiBHEt_3$	-78	THF	22.8	74.3	-
AlH_3	0	THF	14.4	83.1	-
BH_3	0	THF	11.8	83.9	-
9-BBN	-78	THF	10.4	51.4	37
$NaBH_4/CeCl_3$	-78	THF	4.4	89.5	-
L-selectride®	-78	THF	91.1	3.4	4.
L-selectride®	-5	THF	92.7	4.2	2.
L-selectride®	20	THF	92.7	4.8	2.
L-selectride®	-78	Toluene	90.8	5.5	2.
L-selectride®	-78	DEM	54.0	4.0	41
L-selectride®	20	Cyclohexane	74.9	13.9	8.
N-selectride®	-78	THF	97.3	1.6	0.
N-selectride®	-5	THF	94.2	2.6	0.

K-selectride®	-78	THF	96.5	2.0	0.3
K-selectride®	-10	THF	93.6	4.0	0.6
K-selectride®	-78	MTHF	92.2a	6.3	-
LS-selectride®	-78	THF	91.5	4.4	3.2
KS-selectride®	-78	THF	95.5	2.4	0.3
$KBH(Ph)_3$	-78	THF	91.0	4.6	1.7

The reduction is carried out at a temperature from –100°C to 25°C, preferably from –40°C to 25°C, most preferably at about –10°C to 10°C, in a solvent selected from tetrahydrofuran (THF), 2-methyltetrahydrofuran (MTHF), toluene and 1,4-dioxane, most preferably THF.

Such reactions are not restricted to 5β-H sapogen-3-ones. We have made the surprising discovery that K-, L- or N-Selectride® can also be used to reduce 5α-H sapogen-3-ones such as tigogenone, to yield the corresponding 3α-OH, 5α-H sapogenin, e.g. epitigogenin, in a highly stereoselective manner.

In a second aspect of the invention the conversion of 3α-hydroxy-5β-sapogenins to 3β-hydroxy-5β-sapogenins and esters thereof, by a stereospecific inversion reaction. For example, episarsasapogenin can be smoothly converted to sarsasapogenin benzoate by the action of diisopropylazodicarboxylate, triphenylphosphine and benzoic acid (Hughes, Organic Reactions, 1992, 42, 337-400). In a similar fashion epismilagenin can be converted to smilagenin benzoate (Giral, *Ciencia*, 1965, **24**, 89-92). The process is not restricted to benzoate esters but may usefully be employed to make aliphatic, eg, acetate, propionate, n-butyrate, i-butyrate, n-caproate, *i*-caproate, palmitate, substituted aliphatic, e.g. chloroacetate, methoxyacetate, protected amino esters, eg BocAminoglycinate, Bocaminovalinate, CBZaminoglycinate, CBZaminoalinate, or substituted aromatic esters, e.g. chlorobenzoate, nitrobenzoate, dichlorobenzoate etc. (Boc = t-butoxycarbonyl; CBZ = benzyloxycarbonyl).

The process may also proceed *via* an activated form of the 3α,5β-sapogenin, such as the methanesulphonate (mesylate) or *p*-toluenesulphonate, by reaction with an anionic salt of the carboxylic acid (e.g. the sodium, caesium or potassium salt).

Examples

The following Examples illustrate, without limitation, the synthesis of either sarsasapogenin, episarsasapogenin, smilagenin, epismilagenin and epitigogenin utilising selective reductions to control stereochemistry.

Example 1

Synthesis of epismilagenin from smilagenone

Lithium tri-*tert*-butoxyaluminohydride (1M in THF, 99 ml) was added dropwise to a solution of smilagenone (32.0 g, 77.2 mmol) in THF (800 ml) at such a rate that a temperature of 14-16°C was maintained. Once addition was complete the mixture was stirred at room temperature for a further 2 hr. The remaining reducing agent was quenched by the careful addition of ammonium chloride solution (30 g in 400 ml water). The mixture was filtered and the solid washed with DCM (300 ml). The combined filtrates were evaporated and the resiude partitioned between DCM (300 ml) and water (300 ml). The aqueous layer was further extracted with DCM (2 x 300 ml). The combined organics were dried ($MgSO_4$) and evaporated to afford epismilagenin as a white solid (25.7 g).

Example 2

Synthesis of smilagenin from smilagenone with L-Selectride® at –10°C

Smilagenone (657 g) was dissolved in tetrahydrofuran (4000 ml) and the solution purged with nitrogen and cooled to provide an internal temperature of *ca.*-10°C. L-Selectride® (2400 ml 1M in THF) was added over *ca.* 50 minutes and stirred for 90 minutes. A solution of citric acid (600 g) in water (2000 ml) was added slowly, maintaining the temperature below 0°C. The mixture was allowed to warm to ambient temperature and stirred for 30 minutes. The aqueous layer was separated and extracted with dichloromethane (2000 ml) and the layers separated. The aqueous layer was extracted with dichloromethane (1500 ml). The combined organic extracts were washed with water (4000 ml) and dried over $MgSO_4$. The organic extracts were evaporated to dryness to yield smilagenin.

Example 3

Synthesis of smilagenin from smilagenone with K-Selectride® at –15°C

K-Selectride® (1600 ml; 1M in THF) was added the solution of smilagenone (500 g) in THF (3500 ml) at *ca.* –15°C under an atmosphere of nitrogen. The reaction mixture was stirred at this temperature for 30 minutes and quenched with aqueous citric acid (393 g in 1300 ml water), maintaining the internal temperature at *ca.* 0°C. The mixture was warmed to ambient temperature and the THF evaporated at atmospheric pressure until a solid precipitated. The solid was filtered off and dried at the pump.

The solid was dissolved in DCM (6000 ml), dried ($MgSO_4$) and evaporated to a white solid, which was recrystallised from IPA (5000 ml) to afford smilagenin.

Example 4

Synthesis of smilagenin from smilagenone with N-Selectride® at –78°C

N-Selectride® (0.64 ml, 1M in THF) was added to a solution of smilagenone (206 mg) in THF (10 ml) over 10 minutes at –78°C. The mixture was stirred and quenched with 10% aqueous citric acid (2 g in 20 ml water) and the product extracted into DCM (2 x 50 ml), dried (MgSO4) and evaporated to a colourless oil. The oil was taken up in acetone (20 ml) and water (50 ml) added. The precipitate was collected by filtration and dried to afford smilagenin (200 mg, 97%).

Example 5

Synthesis of episarsasapogenin from sarsasapogenin via sarsasapogenone

Sarsasapogenin (1140 g) was dissolved in dichloromethane (3430 ml). A solution of sodium carbonate (166 g in 1700 ml of water) was added, followed by sodium bromide (291 g) and TEMPO (3.7 g). The reaction mixture was cooled to 0°C and a solution of sodium hypochlorite (2311 ml) and sodium hydrogen carbonate (217 g) in water (2000 ml) was added over 4 hours, maintaining the temperature at *ca.* 0°C. The mixture was allowed to warm to ambient temperature and stirred

overnight. A solution of potassium hydrogen sulphate (467 g) in water (1350 ml) was added drop-wise to the well-stirred reaction mixture (CARE! CO_2 evolution). The layers were separated and the lower organic phase filtered through Hyflo. The Hyflo was washed with DCM (3000 ml). The layers were separated and the aqueous phase extracted with DCM (2500 ml). The organic phase was washed with an aqueous solution of potassium hydrogen sulphate (450 g) and potassium iodide (50 g), followed by an aqueous solution of sodium thiosulphate (570 g). The organic layer was separated and evaporated under reduced pressure to afford a solid, which was dried under high vacuum to furnish sarsasapogenone (1110 g, 97%).

Lithium tri-*tert*-butoxyaluminohydride (1M in THF, 472 ml) was added to a cooled solution of sarsasapagenone (40.0 g, 96.5 mmol) in THF (1 L) at such a rate that a temperature of 14-16°C was maintained. Once the addition was complete the mixture was stirred at room temperature for a further 2 hr. The mixture was quenched by the careful addition of sodium sulphate solution. The mixture was filtered though Celite, and the solvents were removed *in vacuo*. This mixture was partitioned between DCM (1 L) and 1:1 sat. brine water. The aqueous layer was further extracted with DCM (500 ml). The combined organics were dried over sodium sulphate. The solvent was removed *in vacuo* to afford sarsasapogenone as a colourless solid.

Example 6

Synthesis of epitigogenin from tigogenin via tigogenone

Tigogenin (8 g) was suspended in acetone (400 ml) and the solution warmed to effect dissolution and re-cooled to room temperature. A solution of chromium oxide (1.92 g) in water (2.8 ml) was cooled to 0°C. and conc. sulphuric acid (1.68 ml) was added followed by water (5.6 ml). This oxidation mixture was added at 0°C to the acetone solution containing tigogenin. The mixture was allowed to warm to room temperature and stirred for approximately 1 hour. The reaction was quenched with water (400 ml) and stirred for a further 5 to 10 minutes. The mixture was filtered and the solid residues dried in a vacuum oven overnight at 55°C to afford crude tigogenone (6.8 g) as a pale green solid. A sample (2.8 g) was dissolved in dichloromethane and passed through a small pad

of silica and washed through with chloroform to remove the green colour to afford the pure tigogenoneas a white crystalline product (1.5 g).

Crude tigogenone (4 g, 1.2 mmol) was dissolved in dry THF (150 ml) and cooled to -78°C. A solution of K-selectride® (1M in THF, 19.2 ml) was added dropwise over 5 minutes and the reaction left stirring for 2 hours. The reaction was quenched with water at -78°C, and allowed to warm to room temperature. The product was extracted into diethyl ether and washed with brine (3 x). Evaporation of the dried organic layer ($MgSO_4$) afforded a white solid, which was purified by recrystallisation from acetone (800 ml) to afford the pure epitigogenin (2 g).

Example 7

Synthesis of smilagenin benzoate from epismilagenin

A solution of diisopropylazodicarboxylate (0.81 g, 4.0 mmol) in dry THF (2 ml) was added to a stirred solution of epismilagenin (0.83 g, 2.0 mmol), triphenylphosphine (1.05 g, 4.0 mmol) and benzoic acid (0.49 g, 4.0 mmol) in dry THF (20 ml). The mixture was stirred at room temperature and monitored by TLC. After 2h all the starting material had been consumed. The solvent was removed *in vacuo*, the residual syrup dissolved in ether (30 ml) and the solution washed with aqueous saturated sodium hydrogen carbonate (25 ml). The organic layer was dried over $MgSO_4$ and passed down a short silica pad, the pad being washed with ether. The combined washings and filtrate were concentrated in vacuo to afford smilagenin benzoate as a white solid.

Example 8

Synthesis of sarsasapogenin benzoate from episarsasapogenin

A solution of diisopropylazodicarboxylate (0.81 g, 4.0 mmol) in dry THF (2 ml) was added to a stirred solution of episarsasapogenin (0.83 g, 2.0 mmol), triphenylphosphine (1.05 g, 4.0 mmol) and benzoic acid (0.49 g, 4.0 mmol) in dry THF (20 ml). The mixture was stirred at room temperature and monitored by TLC. After 2h all the starting material had been consumed. The solvent was removed *in vacuo*, the residual syrup dissolved in ether (30 ml) and the solution

washed with aqueous saturated sodium hydrogen carbonate (25 ml). The organic layer was dried over $MgSO_4$ and passed down a short silica pad, the pad being washed with ether. The combined washings and filtrate were concentrated *in vacuo* to afford sarsasapogenin benzoate as a white solid.

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CLAIMS

1. A stereospecific method of preparing a sapogenin which comprises reducing a sapogen-3-one using as reducing agent: a selectride; an organo-aluminium hydride; a borane; hydrogen and a catalyst; or an alkali metal borohydride and cerium halide.

2. A method according to claim 1, wherein the reducing agent is a selectride and the sapogenin obtained is predominantly a 3β-sapogenin.

3. A method according to claim 2, wherein the selectride is lithium tri-sec-butylborohydride.

4. A method according to claim 1, wherein the reducing agent is an organo-aluminium hydride or hydrogen/platinum and the sapogenin obtained is predominantly a 3α-sapogenin.

5. A method according to claim 4, wherein the organo-aluminium hydride is lithium tri-*tert*-butoxyaluminium hydride.

6. A method according to any one of claims 2 to 5, wherein the molar ratio of the predominant sapogenin obtained, to the alternative 3-epimer, is at least about 10:1.

7. A method according to claim 6, wherein the ratio is at least about 15:1.

8. A method according to claim 1, wherein the sapogen-3-one starting material has a 5β hydrogen atom.

9. A method according to claims 3 and 5, wherein the organic solvent is selected from tetrahydrofuran, diethyl ether, toluene, *tert*-butyl methyl ether, diethoxymethane, 1,4-dioxan, 2-methyltetrahydrofuran and any mixture thereof.

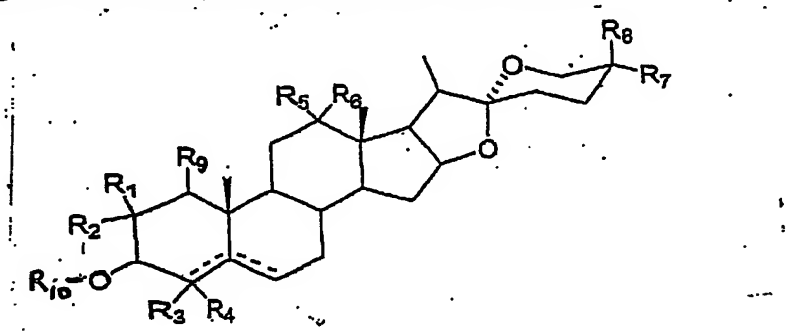
10. A method according to claim 9, wherein the organic solvent consists essentially of tetrahydroforan.

11. A method according to claim 9, wherein the organic solvent consists essentially of toluene.

12. A method according to claim 9, wherein the organic solvent consists essentially of 1,4-dioxan.

13. A method according to claim 9, wherein the organic solvent consists essentially of 2-methyltetrahydrofuran.

14. A method according to any one of the proceeding claims, wherein the desired sapogenin is a compound of general formula (I):



wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are, independently of each other, H, C_{1-4}–alkyl, OH or OR, where R = C_{6-12}–aryl or C_{1-4}–alkyl; or R_5 and R_6 together represent a protected carbonyl group;

---- signifies an optional double bond;

the stereochemistry at the 3 position carbon centre can be either R or S;

and R_{10} is H or any organic ester group (which includes aliphatic and aminoacid esters);

including all stereoisomers thereof and racemic mixtures where the stereochemistry of general formula I permits, their physiologically acceptable prodrugs and salts.

15. A method according to claim 14, wherein the sapogenin is selected from sarsasapogenin, episarsasapogenin, smilagenin and epismilagenin.

16. A method according to any one of the preceding claims, wherein a sapogenin initially formed is subsequently converted to a pro-drug form thereof or to another physiologically acceptable form thereof.

17. A method for the conversion of a 3α,5β-sapogenin into the corresponding 3β,5β-sapogenin by an inversion process *via* an ester derivative.

ABSTRACT

STEREOSPECIFIC SYNTHESIS OF SAPOGENINS

A method of stereospecifically preparing a sapogenin which comprises reducing a sapogen-3-one using as reducing agent: an alkylborohydride; an organo-aluminium hydride; a borane; hydrogen and a catalyst; or an alkali metal borohydride and cerium halide.